IN THE SPECIFICATION

Please replace the paragraph beginning on page 13, line 4 with the following amended paragraph:

Another method of establishing percent homology in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated the "Match" value reflects "sequence homology." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the following internet address: ~~http://www.~~ncbi.nlm.gov/cgi-bin/BLAST.

Please replace the paragraph beginning on page 3, line 5 with the following amended paragraph:

Recently, ~~TaqMan~~ TAQMAN fluorogenic 5' nuclease assays have been used to detect WNV in CSF specimens. Briese et al., *The Lancet* (2000) 355:1614-1615; Lanciotti et al., *J. Clin. Microbiol.* (2000) 38:4066-4071. Lanciotti et al., *J. Clin. Microbiol.* (2001) 39:4506-4513 describes the use of nucleic acid sequence-based amplification (NASBA) for detecting WNV.

This amplification technique employs three enzymes, reverse transcriptase, T7 RNA polymerase and RNase H and the final amplification product is single-stranded RNA with a polarity opposite of the target. The amplified RNA product can be detected using a target-specific capture probe bound to a substrate, in combination with a labeled detector probe. Alternatively, amplified RNA can be specifically detected in real-time using molecular beacon probes in the amplification reaction.

Please replace the paragraph beginning on page 3, line 20 with the following amended paragraph:

The present invention is based on the development of a sensitive, reliable nucleic acid-based diagnostic test for the detection of WNV in biological samples, particularly blood samples, from potentially infected subjects. The techniques described herein utilize extracted sample nucleic acid as a template for amplification of conserved genomic regions of the WNV sequence using transcription-mediated amplification (TMA), as well as in a 5' nuclease assay, such as the ~~TaqMan~~™ TAQMAN real-time PCR technique. The methods allow for the detection of as few as 10 copies of the target WNV sequence in viremic samples. Moreover, the methods described herein provide for a one-pot analysis wherein captured sample nucleic acids can be subjected to amplification and detection in the same container. Using the methods of the invention, infected samples can be identified and excluded from the blood supply for transfusion, as well as for the preparation of blood derivatives.

Please replace the paragraph beginning on page 5, line 27 with the following amended paragraph:

In certain embodiments, the amplifying comprises RT-PCR, transcription-mediated amplification (TMA) or ~~TaqMan~~™ TAQMAN real-time PCR, or a combination thereof.

Please replace the paragraph beginning on page 5, line 29 with the following amended paragraph:

In additional embodiments, the amplifying comprises ~~TaqMan™~~ TAQMAN real-time PCR using the sense primer and the antisense primer and detecting is done using at least one probe comprising a detectable label.

Please replace the paragraph beginning on page 15, line 29 with the following amended paragraph:

As used herein, the term "probe" or "oligonucleotide probe" refers to a structure comprised of a polynucleotide, as defined above, that contains a nucleic acid sequence complementary to a nucleic acid sequence present in the target nucleic acid analyte. The polynucleotide regions of probes may be composed of DNA, and/or RNA, and/or synthetic nucleotide analogs. When an "oligonucleotide probe" is to be used in a 5' nuclease assay, such as the ~~TaqMan™~~ TAQMAN real-time PCR technique, the probe will contain at least one fluorescer and at least one quencher which is digested by the 5' endonuclease activity of a polymerase used in the reaction in order to detect any amplified target oligonucleotide sequences. In this context, the oligonucleotide probe will have a sufficient number of phosphodiester linkages adjacent to its 5' end so that the 5' to 3' nuclease activity employed can efficiently degrade the bound probe to separate the fluorescers and quenchers. When an oligonucleotide probe is used in the TMA technique, it will be suitably labeled, as described below.

Please replace the paragraph beginning on page 18, line 23 with the following amended paragraph:

As noted above, the present invention is based on the discovery of novel diagnostic methods for accurately detecting the presence of West Nile virus (WNV) in a biological sample. The methods can be used to detect WNV in a biological sample from

any vertebrate species susceptible to the virus. The methods rely on sensitive nucleic acid-based detection techniques that allow identification of WNV target nucleic acid sequences in samples containing small amounts of virus. The methods are particularly useful for detecting WNV in blood samples, including without limitation, in whole blood, serum and plasma. The methods can be used to diagnose WNV infection in a subject, as well as to detect WNV contamination in donated blood samples. Thus, aliquots from individual donated samples or pooled samples can be screened for the presence of WNV and those samples or pooled samples contaminated with WNV can be eliminated before they are combined. In this way, a blood supply substantially free of WNV contamination can be provided. By "substantially free of WNV" is meant that the presence of WNV is not detected using the assays described herein, preferably using the ~~TaqMan~~™ TAQMAN fluorogenic 5' nuclease assays described in the examples. Normally, then, a sample will be considered "substantially free of WNV" when less than 5 copies/ml of WNV target nucleic acid are present, preferably less than 3 copies/ml and even more preferably less than 1 copy/ml.

Please replace the paragraph beginning on page 23, line 3 with the following amended paragraph:

The magnetic beads or particles can be produced using standard techniques or obtained from commercial sources. In general, the particles or beads may be comprised of magnetic particles, although they can also include other magnetic metal or metal oxides, whether in impure, alloy, or composite form, as long as they have a reactive surface and exhibit an ability to react to a magnetic field. Other materials that may be used individually or in combination with iron include, but are not limited to, cobalt, nickel, and silicon. A magnetic bead suitable for use with the present invention includes magnetic beads containing poly dT groups marketed under the trade name ~~Sera-Mag~~™ SERA-MAG magnetic oligonucleotide beads by Seradyn, Indianapolis, IN.

Please replace the paragraph beginning on page 29, line 11 with the following amended paragraph:

The fluorogenic 5' nuclease assay, known as the ~~TaqMan~~™ TAQMAN real-time PCR assay (see, e.g., Holland et al., *Proc. Natl. Acad.Sci. USA* (1991) 88:7276-7280), is a powerful and versatile PCR-based detection system for nucleic acid targets. Hence, primers and probes derived from conserved regions of the WNV genome described herein can be used in ~~TaqMan~~™ TAQMAN real-time PCR analyses to detect the presence of WNV in a biological sample. Analysis is performed in conjunction with thermal cycling by monitoring the generation of fluorescence signals. The assay system dispenses with the need for gel electrophoretic analysis, and has the capability to generate quantitative data allowing the determination of target copy numbers. For example, standard curves can be produced using serial dilutions of previously quantified viral suspensions of WNV. A standard graph can be produced with copy numbers of each of the panel members against which sample unknowns can be compared.

Please replace the paragraph beginning on page 30, line 2 with the following amended paragraph:

The amplification products can be detected in solution or using solid supports. In this method, the ~~TaqMan~~™ TAQMAN probe is designed to hybridize to a target sequence within the desired PCR product. The 5' end of the ~~TaqMan~~™ TAQMAN probe contains a fluorescent reporter dye. The 3' end of the probe is blocked to prevent probe extension and contains a dye that will quench the fluorescence of the 5' fluorophore. During subsequent amplification, the 5' fluorescent label is cleaved off if a polymerase with 5' exonuclease activity is present in the reaction. Excision of the 5' fluorophore results in an increase in fluorescence which can be detected. Representative labeled probes include the probes of SEQ ID NOS:36, 39, 44 and 49.

Please replace the paragraph beginning on page 30, line 11 with the following amended paragraph:

For a detailed description of the ~~TaqMan~~™ TAQMAN fluorogenic 5' nuclease assay, reagents and conditions for use therein, see, e.g., Holland et al., *Proc. Natl. Acad. Sci, U.S.A.* (1991) 88:7276-7280; U.S. Patent Nos. 5,538,848, 5,723,591, and 5,876,930, all incorporated herein by reference in their entireties.

Please replace the paragraph beginning on page 36, line 28 with the following amended paragraph:

The lysis buffer used was from Organon-Teknika (Durham, NC). This lysis buffer contained guanidinium isothiocyanate to solubilize proteins and inactivate RNases and DNases, and Triton X-100. The detergent Triton X-100 further facilitated the process of solubilization and disintegration of cell structure and nuclear proteins, thus releasing nucleic acid. In particular, the cultured WNV was serially diluted using serum from Seracure (Ocean Side, CA). Pre-aliquoted 9.0 ml of the lysis reagent was used to extract RNA from 0.5 ml of the WNV-positive serum (10^5/ml). Magnetized silica (~~MagPrep~~ MAGPREP particles, Novagen, WI) was substituted for regular silica and magnetic base was used to capture the nucleic acid-bound silica particles, thus eliminating centrifugations required to sediment regular silica particles. The bound nucleic acids were eluted in 50 µl of 10 mM Tris pH 8.0 containing 1 mM EDTA. Following nucleic acid isolation, the presence of WNV was determined by performing ~~TaqMan~~™ TAQMAN RT-PCR, as described below.

Please replace the paragraph beginning on page 37, line 13 with the following amended paragraph:

Although use of magnetized silica greatly facilitates rapid and easy handling during the washing and elution steps, isolation of nucleic acid is still laborious and time

consuming. Therefore one-step capture of specific nucleic acid target from plasma or serum using magnetic beads was used. In order to make this applicable for a wide variety of viral nucleic acid capture tests, generic magnetic beads coupled with oligo dT were used. ~~Sera-Mag~~ SERA-MAG magnetic oligo (dT) beads (Seradyn, Indianapolis, IN) with an oligo dT length of about 14 bps, were used in combination with Capture oligonucleotides containing from 21-24 poly A's at the 3' end contiguous with the WNV-specific sequence used (designated at the end of the sequence specified below).

Please replace the paragraph beginning on page 38, line 1 with the following amended paragraph:

The capture primers were tested individually or in combination, to capture 100 copies/ml of WNV RNA. Following capture, the beads were washed three times with a wash buffer of 10 mM Hepes (pH 7.5), 0.5% NP-40 containing 0.3 M NaCl. The beads with the captured nucleic acid were suspended in 100 µl of ~~TaqMan~~™ TAQMAN one-step RT-PCR reagent and transferred to a ~~TaqMan~~™ TAQMAN RT-PCR microtiter plate for detection by ~~TaqMan~~™ TAQMAN PCR as described below. Several oligonucleotide combinations were efficient at capturing WNV as detected by the ~~TaqMan~~™ TAQMAN PCR assay.

Please replace the paragraph beginning on page 39, line 2 with the following amended paragraph:

Detection and Quantitation of WNV Nucleic Acid by ~~TaqMan~~™ TAQMAN PCR

~~TaqMan~~™ TAQMAN real-time PCR technology was used for amplifying the captured target as DNA. For this amplification, three sets of oligonucleotides were derived from conserved regions within the capsid (VWNVA1-VWNVA3), 3'UTR (VWNVA4-VWNVA6), and NS1/NS2 region (VWNVA7-VWNVA9) of the WNV genome. The primer and probe sets were as follows (the numbering indicated at the end

of the sequence corresponds to the position within the WNV genome, relative to NCBI accession number AF196835):

Please replace the paragraph beginning on page 39, line 32 with the following amended paragraph:

Reagents for the ~~TaqMan~~™ TAQMAN real-time PCR analysis were obtained from Applied Biosystems, Foster City, CA. The nucleic acid from Example 1(a) in a 47 µl volume was used in the ~~TaqMan~~™ TAQMAN real-time PCR assay in a total volume of 100 µl by adding 2X one-step RT-PCR master mix reagent containing 0.4 pmol of the probe. Alternatively, 100 µl of the 1X one-step RT-PCR master mix reagent containing 1 pmol of each of the amplification primers, and 0.4 pmol of the probe, was added to target captured on the magnetic beads and the suspension transferred to a ~~TaqMan~~™ TAQMAN microtiter plate. The reaction conditions were 48 °C for 30 min for the RT reaction, 10 min at 95 °C to activate the enzyme followed by 50 cycles of 30 seconds at 95 °C, alternating with 1 min at 60 °C in an ABI 7900 Sequence Detector. The two sets of oligonucleotides described above were used.

Please replace the paragraph beginning on page 40, line 9 with the following amended paragraph:

Using the protocol of target with capture primers and ~~TaqMan~~™ TAQMAN RT-PCR technology, as few as 10 copies of the target could be detected.

Please replace the paragraph beginning on page 40, line 22 with the following amended paragraph:

The WNV RNA isolated by binding to silica was amplified in the ~~TaqMan~~™ TAQMAN real-time PCR assay and detected using the methods, primers and probes

described above. Typically, signals from samples realized <45 cycles at a threshold of >0.2 were considered positive. Table 1 details the results.

Please replace the paragraph beginning on page 41, line 19 with the following amended paragraph:

In additional experiments, reagents from Invitrogen Corporation (Carlsbad, CA) were used. In particular, these experiments used the Invitrogen ~~Superscript III Platinum~~ SUPERSCRIPT III PLATINUM one-step ~~Q~~quantitative RT-PCR system. The nucleic acid from Example 1(a) was suspended in 100 µl of reaction mix containing 2 µl of ~~Superscript IIIRT/Platinum~~ SUPERSCRIPT III RT PLATINUM Taq mix, 50 µl of 2X ~~R~~reaction mix, 4 mM $MgSO_4$, 2 µl of ROX, 1 pmol of amplification primers and 0.25 pmol of the probes. The suspended beads were transferred to a ~~TaqMan~~™ TAQMAN microtiter plate. The reaction conditions were 50°C for 15 min for the RT reaction, followed by 95°C for 2 min to denature the Taq polymerase antibody, followed by 50 cycles of alternating incubations at 95°C for 15 seconds, and 60°C for 1 min. Using the protocol of target capture primers and ~~TaqMan™ superscript~~ TAQMAN SUPERSCRIPT RT-PCR technology, 100% detection of 7.5 copies/ml (Cps/ml) of WNV RNA was observed (see, Table 2).